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Occurrence of *Listeria* spp. in Retail Meat and Dairy Products in the Area of Addis Ababa, Ethiopia

Firehiwot Abera Derra,¹ Susanne Karlsmose,² Dharam P. Monga,³ Abebe Mache,³ Christina Aaby Svendsen,² Benjamin Félix,⁴ Sophie A. Granier,⁴ Abera Geyid,¹ Girum Taye,¹ and Rene S. Hendriksen²

Abstract

Background: Listeriosis, a bacterial disease in humans and animals, is mostly caused by ingestion of *Listeria monocytogenes* via contaminated food and/or water, or by a zoonotic infection. Globally, listeriosis has in general a low incidence but a high case fatality rate.

Objective: The objective of this study was to investigate the occurrence, antimicrobial profiles, and genetic relatedness of *L. monocytogenes* from raw meat and dairy products (raw milk, cottage cheese, cream cake), collected from the capital and five neighboring towns in Ethiopia.

Methods: Two hundred forty food samples were purchased from July to December 2006 from food vendors, shops, and supermarkets, using a cross-sectional study design. *L. monocytogenes* were isolated and subjected to molecular serotyping. The genetic relatedness and antimicrobial susceptibility patterns were investigated using pulsed-field gel electrophoresis (PFGE) and minimum inhibitory concentration determinations.

Results: Of 240 food samples tested, 66 (27.5%) were positive for *Listeria* species. Of 59 viable isolates, 10 (4.1%) were *L. monocytogenes*. Nine were serotype 4b and one was 2b. Minimum inhibitory concentration determination and PFGE of the 10 *L. monocytogenes* isolates showed low occurrence of antimicrobial resistance among eight different PFGE types.

Discussion and Conclusions: The findings in this study correspond to similar research undertaken in Ethiopia by detecting *L. monocytogenes* with similar prevalence rates. Public education is crucial as regards the nature of this organism and relevant prevention measures. Moreover, further research in clinical samples should be carried out to estimate the prevalence and carrier rate in humans, and future investigations on foodborne outbreaks must include *L. monocytogenes*.

Introduction

LISTERIA MONOCYTOGENES is an important cause of diseases in both animals and humans. The incidence of human listeriosis is low (0.1–11.3 cases per million inhabitants), but it is increasing in Europe (Goulet *et al.*, 2008). The mortality rate is reported to be 20–30% (Swaminathan and Gerner Smidt, 2007).

In Ethiopia, few findings of *L. monocytogenes* have been reported, possibly due to lack of attention or resources (Molla *et al.*, 2004).

The objective was to investigate the occurrence and prevalence of *Listeria* spp., the antimicrobial resistance, and clonal

relatedness among *L. monocytogenes* in samples of raw meat and dairy products from Addis Ababa and neighboring towns in Ethiopia.

Methods

A cross-sectional study was conducted in 2006 at the Public Health Food Microbiology Laboratory of Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa, Ethiopia.

A total of 240 food samples—all different samples of raw meat, unpasteurized milk, cottage cheese, and cream cake—were purchased randomly from different retail markets,

¹Food Microbiology Section, Food Science and Nutrition Research Directorate, Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia.

²WHO Collaborating Centre for Antimicrobial Resistance in Food-borne Pathogens and EU Reference Laboratory for Antimicrobial Resistance, National Food Institute, Technical University of Denmark, Kingsly Lyngby, Denmark.

³Microbiology, Immunology, and Parasitology Department, Medical Faculty, Addis Ababa University, Addis Ababa, Ethiopia.

⁴Paris-Est University, ANSES, French Agency for Food, Environmental, and Occupational Health and Safety, Laboratory for Food Safety, European Union Reference Laboratory for *Listeria monocytogenes*, Maisons-Alfort, France.

shops, supermarkets, and food vendors in suburbs of Addis Ababa and neighboring towns from July to December 2006.

Samples of 500 g or 500 mL of each food category were purchased and examined according to the standard International Dairy Federation method (IDF, 1990).

Based on biochemical testing, 66 isolates were *Listeria* spp., of which 59 remained viable after prolonged storage at -20°C in trypticase soy broth (TSB) supplemented with 10% glycerol. The isolates were then subjected to speciation by polymerase chain reaction assay (PCR) (Huang *et al.*, 2007), genotyped by pulsed-field gel electrophoresis (PFGE) (Graves and Swaminathan, 2001), and antimicrobial susceptibility testing for minimum inhibitory concentration (MIC) determination (Aarestrup *et al.*, 2007).

The isolates were identified at the species level utilizing a multiplex PCR previously described (Huang *et al.*, 2007). Modifications to the method included one pair of oligonucleotide primers: 5'-CTCCCACATTGGTGCTACTCA-3' and 5'-GTTGCTGCGAACTTAACCTCA-3'. Amplicon sizes of 453, 373, 609, 420, 465, and 289 bp, respectively, identified each of the species *L. monocytogenes*, *L. seeligeri*, *L. welshimeri*, *L. grayi*, *L. ivanovii*, and *L. innocua*.

The 10 *L. monocytogenes* isolates were subjected to *L. monocytogenes*-specific serotyping utilizing a multiplex PCR assay according to K  rouanton *et al.* (2010). These isolates were genotyped by PFGE using *Apa*I and *As*cI with optimization set of 1% (Graves and Swaminathan, 2001). Comparative analysis based on unweighted pair group method using arithmetic averages was performed using Bionumerics software version 4.6 (Applied Maths, Sint-Martens-Latem, Belgium).

MIC determination was performed on the 10 *L. monocytogenes* isolates using Mueller-Hinton broth supplemented with 5% lysed bovine blood and a dehydrated panel from TREK Diagnostic Systems Ltd. (East Grinstead, England) with an incubation period of 24 h. Antimicrobials and epidemiological cut-off values used were based on guidelines for *L. monocytogenes* from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) when available (i.e., for erythromycin, penicillin, tetracycline and sulfamethoxazole + trimethoprim). For the remaining antimicrobials—cefoxitin, chloramphenicol, ciprofloxacin, florfenicol, gentamicin, spectinomycin, streptomycin, sulfamethoxazole, tiamulin, and trimethoprim—they were utilized as described (Aarestrup *et al.*, 2007).

Results

Sixty-six (27.5%) of the 240 food samples were positive by conventional culture technique for *Listeria* spp., and 59 isolates viable after prolonged storage positives were raw meat 62.7%, raw milk 8.5%, cottage cheese 6.8%, and cream-cake samples 22.0%, respectively.

The species-specific PCR revealed that 10 (17%) of the 59 isolates were *L. monocytogenes* and 49 (83%) isolates belonged to the species *innocua*. *L. monocytogenes* was isolated from four (6.8%), two (3.4%), one (1.7%), and three (5.1%) samples of raw meat, raw milk, cottage cheese, and cream cake, respectively. Nine of those belonged to serotype 4b and one (isolated from cream cake) to serotype 2b.

MIC determination of the 10 *L. monocytogenes* isolates showed low frequency of antimicrobial resistance. Intrinsic resistance to cefoxitin was not considered relevant; only resistance to the following five antimicrobials was observed:

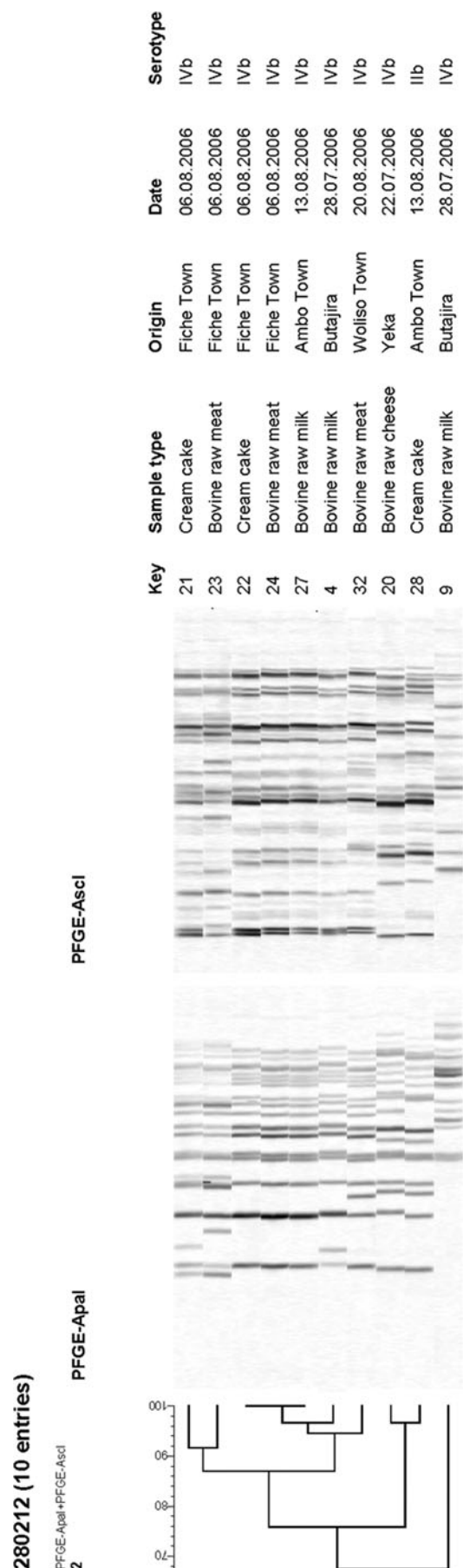


FIG. 1. Dendrogram showing the genotypic relatedness among *Listeria monocytogenes* isolates based on *Apa*I/*As*cI pulsed-field gel electrophoresis (PFGE) fingerprints. Similarity value was calculated using the Dice coefficient, and tolerance and optimization were set at 1%. Profiles are grouped together according to the unweighted pair group method using arithmetic averages.

ciprofloxacin ($n=3$), gentamicin ($n=1$), penicillin ($n=1$), tiamulin ($n=10$), and sulfamethoxazole ($n=1$).

The 10 *L. monocytogenes* isolates rendered eight different PFGE types. One cluster was identified that contained three indistinguishable isolates, of which two originated from cream cheese sampled at different towns and dates, and one was isolated from raw meat on the same day and town as one of the cream-cake isolates (Fig. 1).

Discussion

This study as well as the previous Ethiopian study by Molla *et al.* (2004) showed that *Listeria* species exist in the Ethiopian food production system. Molla *et al.* (2004) detected all species of *Listeria*, whereas in this study only *L. monocytogenes* and *L. innocua* were found. The reason might relate to the limited number of collected samples. We found *L. innocua* to be the most predominant species (83%), which is in contrast to what has previously been reported in the United States by Zhang *et al.* (2007), who observed a prevalence rate of 65% in the same food categories as investigated here. The distribution of *L. monocytogenes* in indicated food types was compared with other studies, and similar findings were observed (Zhang *et al.*, 2007).

All 10 isolates in this study were resistant to tiamulin, a veterinary-approved antimicrobial usually described as effective against *L. monocytogenes* for the last few decades. A previous study has already shown high levels of MICs to tiamulin in *L. monocytogenes* originating from animals (Aarestrup *et al.*, 2007), which contributes to doubting the efficacy of tiamulin against *L. monocytogenes*. More worrisome is the resistance to gentamicin, which is recommended, in combination with penicillins, as first-line treatment of listeriosis in humans. A similar finding was reported in Botswana by Morobe *et al.* (2009).

The diversity and cluster observed for the strains 22, 24, and 27 (Fig. 1) suggests a low clonal relatedness among the isolates in contrast to a normally high clonality of the species. Serotypes 4b and 2b isolated here have previously been associated with human foodborne illness (Kathariou, 2002). Unfortunately, it has not been possible to compare the PFGE patterns of serotype 4 in this study with isolates belonging to epidemic clones (Kathariou, 2002).

This study highlights the presence of *L. monocytogenes* and *L. innocua* in raw food items from Ethiopia. Considering the risk of contracting listeriosis due to the practice of consuming raw food such as raw meat and unpasteurized and fermented flavored milk, especially in association with poor knowledge of good manufacturing practice and of handling the products, we suggest that the Ethiopian authorities liaise with the World Health Organization to assist the implementation of the technology for an integrated production-to-consumption approach to food safety. Such an approach relies mainly on the hazard analysis and critical control point system and good manufacturing practice (www.who.int/foodsafety/fs_management/en/). Furthermore, we suggest that the Ethiopian authorities provide educational information for the Ethiopian consumers by promoting the World Health Organization's "Five Keys to Safer Food" (www.who.int/foodsafety/fs_management/en/).

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:

Firehiwot Abera Derra, MSc

Food Microbiology Section

Food Science and Nutrition Research Directorate

Ethiopian Health and Nutrition Research Institute (EHNRI)

Gulele subcity of Addis Ababa, Kebele 08

Addis Ababa, Ethiopia

E-mail: firehiwot_a@yahoo.com